Synthesis of Thymidine Dimers Containing Internucleoside Sulfonate and Sulfonamide Linkages

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Received August 20, 1990 (Revised Manuscript Received March 26, 1992)

Summary: Novel chemistry is reported for the formation of internucleoside sulfonate and sulfonamide linkages.

The antisense oligonucleotide approach to gene regulation offers an exquisitely specific chemotherapeutic strategy to disrupt pathogenic diseases such as viral, bacterial, and parasitic infections as well as cancer.[2] This specificity is inherently built into the antisense oligonucleotide via the information contained in the nucleotide sequence and theoretically allows the selective inhibition of target gene expression through specific base pairing (in a Watson–Crick sense) or hybridization to the target sequence thus selectively disrupting only the utilization (transcription, translation and replication) of the target gene or messenger RNA within the cell.

Considerable attention and laboratory effort has been expended in an attempt to propel antisense agents into the clinic and, while this goal has not been realized, significant advances have been made. In an effort to extend the pharmacological utility of the natural phosphate-linked antisense oligomers, which suffer from poor cellular permeability and rapid degradation through the action of plasma and intracellular nucleases, nonionic oligonucleotide analogs and nuclease-resistant phosphate-based linkers have been developed. The methylphosphonate-,[3] phosphorothioate-,[4] and phosphorodithioate[5]-based oligomers are representative of this approach. We feel, however, that each of these congeners suffers inherent shortcomings which will compromise the effectiveness of any resultant drug. Because of our conviction that the ideal internucleoside linkage should be both achiral (to maximize potency and simplify oligomer synthesis and purification) and nonionic (to facilitate cellular uptake), we embarked upon a program to design oligonucleotides based on a general, easily accessible phosphate isostere which would avoid the difficulties encountered in the earlier generation of linkers.

The close homology between the SO_2 and the PO_2^- moieties in terms of size, bond angles, and bond lengths suggested that such a sulfur-based nonionic oligonucleotide might overcome several of the aforementioned drawbacks of other modified oligonucleotides.[6,7] In addition to the nuclease resistance the sulfonyl-based DNA congeners should share with other oligonucleotide surrogates,[8] these nonionic oligonucleotide analogs should also have enhanced lipid membrane permeability relative to the charged oligonucleotides which should augment cellular availability. Unlike previously reported classes of nonionic DNA analogs, some of which have been hampered by poor aqueous solubility, the polarity of sulfonyl-based oligomers should improve solubility. While the sulfonamide link is only weakly acidic, an important requirement for cell permeability, this property may confer added solubility to longer sulfonamide-based oligomers over other nonionic linkages. Even without ionization, the added hydrogen bonding capacity of this link should improve solubility of sulfonamide-based oligonucleotides.[9] However, if ionized (potentially in a basic pocket of an enzyme active site), the resulting anionic sulfonamide linkage would undoubtedly be isoelectronic and essentially isosteric with the natural phosphate linkage, although the negative charge would be delocalized over bridging and nonbridging atoms of the sulfonamide linkage. The isoelectronic and isosteric nature of both the sulfonate and sulfonamide linkers may thus be relevant to the potential of these sulfonyl-based oligomers to bind to enzyme active sites, e.g., that of RNase H, an enzyme activity critical for the catalytic destruction of target mRNA.[2c] Although there are no examples of any neutral oligonucleotide that can replace DNA (in a duplex of mRNA–DNA) as a recognition element for ribonuclease H, we feel that no uncharged analogs have been tested which have such a close homology with the natural phosphate link. Finally, the sulfonyl group is achiral, obviating the difficult syntheses and purifications required with some other groups, viz. methylphosphonate and phosphorothioate, to obtain diastereomerically homogeneous material. In this paper we wish to report the synthesis of thymidine dimers containing either a 3′- to 6′-sulfonate or sulfonamide internucleoside linkage.

Our synthetic strategy was based on utilization of the readily available 5′-homologated thymidine sulfonic acids (**1a–b**, Scheme I), which were converted on an XAD-4 column to their more organically soluble *N,N*-diisopropylethylammonium salts.[10] A variety of coupling methodologies were pursued, in model reactions as well as

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(6) Several groups have recently reported research relating to sulfide, sulfone, and sulfonate internucleoside linkages: (a) Musicki, B.; Widlanski, T. S. *J. Org. Chem.* **1990**, *55*, 4231–33. (b) Huang, Z.; Schneider, K. C.; Benner, S. A. *J. Org. Chem.* **1991**, *56*, 3869–3882. (c) Musicki, B.; Widlanski, T. S. *Tetrahedron Lett.* **1991**, *32*, 1267–1270.

(7) Schmidt, M.; Siebert, W. In *Comprehensive Inorganic Chemistry*; Bailar, J. C., Emeléus, H. J., Nyholm, R., Trotman-Dickenson, A. F., Eds.; Pergamon Press: Oxford, UK, 1973; Vol. 2, pp 795–933.

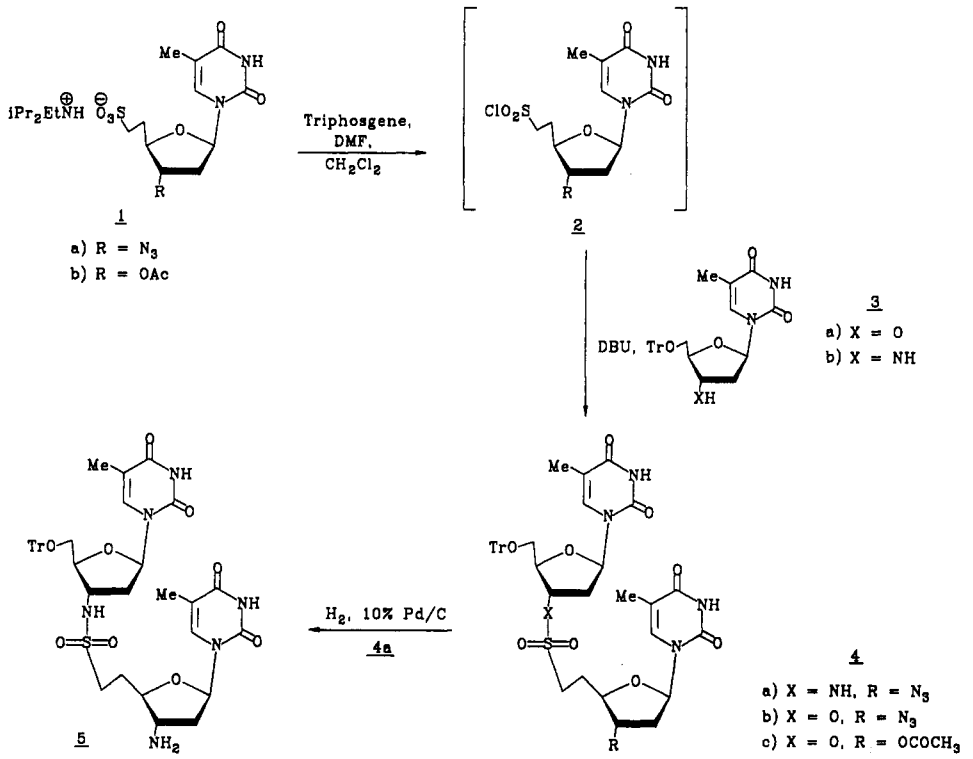
(8) For a general discussion of the stability of the sulfonyl link in a biological setting see: Crooks, P. A. *Sulphur Containing Drugs and Related Organic Compounds—Chemistry, Biochemistry, and Toxicology*; Damani, L. A., Ed.; Ellis Horwood–John Wiley and Sons: London, 1989; Vol. I, Part B.

(9) In a single experiment, we found that approximately 9 mg of detritylated **5** would readily dissolve with stirring in 1.0 mL of 1.0 M triethylammonium bicarbonate buffer at pH 7.5 for a resulting concentration of about 17 mM.

(10) Crooks, P. A.; Reynolds, R. C.; Maddry, J. A.; Rathore, A.; Akhtar, M. S.; Montgomery, J. A.; Secrist, J. A., III. *J. Org. Chem.* Accepted for publication.

0022-3263/92/1957-2983$03.00/0

Scheme I



dimer formation (see Table I).[11,12] Among the model reactions studied were those leading to the isopropyl ester (**6**) of **1a**, the neopentyl ester (**7**) of **1a**, the 3′-*O*-butanesulfonate (**8**) of **3a**, and the 3′-*N*-butanesulfonamide (**9**) of **3b**.[13] Compound **6**, in contrast to reports on the stability of similar compounds, appears to be relatively unstable and is partially cleaved by silica gel chromatography and decomposes completely on sitting at room temperature for an extended period. Furthermore, the isopropyl ester is quantitatively cleaved under reductive conditions (either H_2 (atm)/10% Pd–C or NH_4^+-$HCOO^-$/10% Pd–C). The neopentyl ester **7**, however, appears to be stable to isolation and storage.

Although many reagents have been reported for the synthesis of carboxylate and phosphate esters and amides from the respective acids, application of these reagents to sulfonic acid esters and amides is not necessarily straightforward, leading to an examination of a number of mild approaches to the activation and subsequent derivatization of sulfonic acids (see the Table I). After investigation of a variety of coupling approaches and conditions, we found that it was possible to generate a sulfonyl chloride in high yield under very mild conditions using the reagent triphosgene.[11d] While the sulfonyl chloride could be isolated, better coupling yields were obtained by immediate reaction of it with either **3a**[14] or **3b**.[15] It is notable

Table I. Strategies for Formation of Sulfonates and Sulfonamides[a]

coupling agent	RSO_3H	R′OH or R′NH_2	yield (%)	ref
tipsyl Cl	$BuSO_3Na$	**3a**	75	11a
tipsyl Cl	$BuSO_3Na$	**3b**	26	11a
tipsyl Cl	**1a**	**3a**	60	11a
tipsyl nitrotriazole	**1a**	**3b**	7	11b
tipsyl Cl	**1a**	*i*-PrOH	50	11a
Ph_3P/TfOTf	**1a**	*i*-PrOH	36	11c
Ph_3P/TfOTf	**1a**	**3b**	30	11c
tipsyl Cl	**1a**	$(CH_3)_3CCH_2OH$	34	11a
triphosgene/DMF/pyridine	**1a**	**3b**	52	11d, 15
triphosgene/DMF/pyridine	**1a**	**3a**	65	11d, 15
triphosgene/DMF/DBU	**1b**	**3a**	43	11d,e
triphosgene/DMF/DBU	**1a**	**3b**	65	11d,e, 15
triphosgene/DMF/DBU	**1a**	**3a**	90	11d,e, 15

[a] Unsuccessful methods examined are briefly mentioned in ref 12.

(11) (a) Lohrmann, R.; Khorana, H. G. *J. Am. Chem. Soc.* **1966**, *88*, 829–833. (b) Jones, S. S.; Rayner, B.; Reese, C. B.; Ubasawa, A.; Ubasawa, M. *Tetrahedron* **1980**, *36*, 3075–3085. (c) Hendrickson, J. B.; Hussoin, Md. S. *J. Org. Chem.* **1989**, *54*, 1144–1149. (d) Eckert, H.; Forster, B. *Angew. Chem.* **1987**, *99*, 922 (*Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 894–895). (e) Ono, N.; Yamada, T.; Saito, T.; Tanaka, K.; Kaji, A. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 2401–2404.

(12) Other, unproductive, coupling reactions were attempted and the reagents are listed with appropriate references below. (a) Tipsyl chloride/phenol. (b) Tipsyl chloride/4-nitrophenol. (c) DCC; for preparation of sulfonic anhydrides via DCC see: Khorana, H. G. *Can. J. Chem.* **1953**, *31*, 585–588. (d) $SOCl_2$, $SOCl_2/Bu_4N^+Cl^-$, or $POCl_3$/DMF: March, J. *Advanced Organic Chemistry*; John Wiley and Sons: New York, 1985; p 445 and references cited therein. (e) Me_3NO/triflic anhydride, attempted activation via analogous intermediates of ref 11c. For review of this reagent see: Soderquist, J. A.; Anderson, C. L. *Tetrahedron Lett.* **1986**, *27*, 3961–3962. (f) Ph_3PCl_2 or Ph_3PBr_2: Aizpurua, J. M.; Cossio, F. P.; Palomo, C. *J. Org. Chem.* **1986**, *51*, 4941–4943. (Sulfonyl chloride formation was noted in the Ph_3PCl_2 reaction and the product could be isolated in low yields. However, no coupling was achieved.) (g) Triflic anhydride: Parish, R. C.; Stock, L. M. *Tetrahedron Lett.* **1964**, 1285–1288. (h) $PhOPOCl_2$: Garcia, T.; Arrieta, A.; Palomo, C. *Synth. Commun.* **1982**, *12*, 681–690. (i) *p*-$NO_2PhOCOCl$: Kim, S.; Lee, J. I.; Kim, Y. C. *J. Org. Chem.* **1985**, *50*, 560–565. (j) *N,N,N′,N′*-Tetramethyl(succinimido)uronium tetrafluoroborate; for the preparation and use of this reagent see: Bannwarth, W.; Knorr, R. *Tetrahedron Lett.* **1991**, *32*, 1157–1160.

(13) Satisfactory analytical data were obtained for **6–9** and are available as supplementary material.

(14) Horwitz, J. P.; Urbanski, J. A.; Chua, J. *J. Org. Chem.* **1962**, *27*, 3300–3302.

(15) Synthesized via reduction of the known compound, 3′-azido-5′-tritylthymidine (see: Glinski, R. P.; Khan, M. S.; Kalamas, R. L.; Sporn, M. B. *J. Org. Chem.* **1973**, *38*, 4299–4305), using 10% palladium on activated carbon under atmospheric hydrogen pressure. The paper describing this derivative is in preparation.

that the coupling of the sulfonyl chloride with both **3a** and **3b** is greatly enhanced by the presence of a catalytic amount of DBU, presumably via a previously proposed mechanism.[11e] Three sulfonyl-bridged thymidine dimers **4a–c** were prepared via this method.[16] Additionally, **4a**, the 3'-azido-terminated sulfonamide dimer, was reduced to the 3'-amino derivative using 10% Pd–C in methanol under atmospheric hydrogen to give **5** in very good yield.[17] The amino derivative **5** could potentially be extended by coupling chemistry reported herein.

In summary, novel chemistry has been reported for the formation of both sulfonate and sulfonamide bonds, and this chemistry has been utilized to form thymidine dimers containing both internucleoside sulfonate and sulfonamide linkages. Sulfonamide linked oligomers have never been previously reported, and the method reported herein would allow variation of the oligomer sequence both for sulfonates and sulfonamides. This is a critical advancement if this new linker is to have relevance in the antisense arena. We are presently optimizing reaction conditions for both types of linkages with all of the naturally occurring nucleoside bases and, at the same time, pursuing the synthesis of longer oligomers.

Acknowledgment. This work was supported by the National Institutes of Health, Grant Numbers U01 AI26054 and U01 AI26061.

Supplementary Material Available: Experimental data for **2**, **5** (detritylated), and **6–9** (3 pages). Ordering information is given on any current masthead page.

(16) **General Procedure for the Preparation of 4a–c.** To a solution of **1a** or **1b** (0.56 mmol) in dry CH_2Cl_2 (2 mL) under dry N_2 was added 1 drop of dry DMF followed by the addition of triphosgene (83 mg, 0.28 mmol). The resulting solution was stirred at ambient temperature for 1 h. TLC ($CHCl_3$–MeOH (7:3)) indicated the complete disappearance of **1** with the formation of a single spot on TLC (silica gel, R_f 0.45 in THF) which corresponds to the sulfonyl chloride (**2**: R = N_3) when isolated. To this reaction mixture was added a solution of **3a** or **3b** (0.56 mmol) and DBU (0.09 mL, 0.6 mmol) in dry CH_2Cl_2 (2 mL). The reaction was stirred at ambient temperature for 16 h. The solvent was evaporated under reduced pressure, and the product was purified on a 2-mm silica gel preparative TLC plate ($CHCl_3$–MeOH (9:1)) to yield **4a–c**. **4a**: yield 65%; mp 154–155 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.50 (s, 3 H, CH_3), 1.76 (s, 3 H, CH_3), 1.92–2.14 (m, 2 H, 5''-CH_2), 2.20–2.36 (m, 2 H, 2''-CH_2), 2.42–2.56 (m, 2 H, 2'-CH_2), 2.98–3.18 (m, 2 H, O_2SCH_2), 3.20–3.28 (m, 2 H, 5'-CH_2), 3.68–3.78 (m, 1 H, 4''-CH), 3.86–3.94 (m, 1 H, 4'-CH), 4.06–4.20 (m, 1 H, 3'-CH), 4.28–4.35 (q, 1 H, 3''-CH), 6.05–6.10 (t, 1 H, 1''-CH), 6.14–6.18 (t, 1 H, 1'-CH), 7.23–7.44 (m, 16 H, trityl H and 6 H), 7.51 (s, 1 H, 6 H), 7.70 (bs, 1 H, SO_2NH), 11.34 (bs, 2 H, 2 thymine NH); ^{13}C NMR (Me_2SO-d_6) δ 11.7, 11.9 (2 CH_3's), 26.9 (5''-C), 35.1 (2''-C), 37.6 (2'-C), 48.6 (O_2SC), 52.0 (3'-C), 62.2 (3''-C), 62.6 (5'-C), 80.5 (4''-C), 82.6 (4'-C), 83.2, 83.3 (1'-C and 1''-C), 86.3 (trityl C), 109.4, 109.9 (two 5-C's), 127.0, 127.7, 128.1 (aromatic C), 135.6, 136.1 (two 6-C's), 143.3 (aromatic C), 150.1, 150.2 (two 2-C's), 163.4, 163.5 (two 4-C's); IR (KBr) cm^{-1} 2105 s (N_3), 1690 bs (CO), 1468, 1449, 1271, 1142; FAB-MS (negative mode) m/e 809 (M – H)$^-$. Anal. Calcd for $C_{40}H_{42}N_8O_9S{\cdot}H_2O$: C, 57.96; H, 5.34; N, 13.35. Found: C, 58.11; H, 5.28; N, 13.04. **4b**: yield 90%; mp 125–127 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 2.02–2.20 (m, 2 H, 5''-CH_2), 2.24–2.40, 2.45–2.60 (2 m, 4 H, 2'-CH_2 and 2''-CH_2), 3.22–3.36 (m, 2 H, 5'-CH_2), 3.40–3.60 (m, 2 H, OSO_2CH_2), 3.76–3.84 (m, 1 H, 4''-CH), 4.32–4.40 (m, 1 H, 4'-CH), 5.32–5.40 (m, 1 H, 3'-CH), 6.07–6.12 (t, 1 H, 1'-CH), 6.20–6.24 (t, 1 H, 1''-CH), 7.24–7.42 (m, 15 H, trityl H), 7.46 (1 s, 1 H, 6 H), 7.48 (1 s, 1 H, 6 H), 11.34 (bs, 2 H, 2 NH's); IR (KBr) cm^{-1} 2105 s (N_3), 1692 bs (CO), 1468, 1449, 1365, 1273, 1170; FAB-MS (negative mode) m/e 810 (M – H)$^-$. Anal. Calcd for $C_{40}H_{41}N_7O_{10}S{\cdot}1.5H_2O$: C, 57.27; H, 5.28; N, 11.68. Found: C, 57.28; H, 5.27; N, 11.72. **4c**: yield 43%; mp 139–142 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.28 (s, 3 H, CH_3), 1.76 (s, 3 H, CH_3), 2.06 (s, 3 H, $COCH_3$), 2.10–2.28 (m, 3 H, 5''-CH_2 and 2''-CH), 2.46–2.48 (m, 3 H, 2'-CH_2 and 2''-CH_2), 3.24–3.30 (m, 2 H, 5'-CH_2), 3.28–3.58 (m, 2 H, O_2SCH_2), 3.90–3.98 (mm, 1 H, 4''-CH), 4.18–4.24 (m, 1 H, 4'-H), 5.04–5.12 (m, 1 H, 3''-CH), 5.30–5.38 (m, 1 H, 3'-CH), 6.10–6.15 (t, 1 H, 1'-CH), 6.18–6.23 (t, 1 H, 1''-CH), 7.27–7.40 (m, 15 H, trityl H), 7.46 (s, 1 H, 6 H), 7.54 (s, 1 H, 6 H), 11.35 (s, 1 H, NH), 11.40 (s, 1 H, NH); IR (KBr) cm^{-1} 1692 bs (CO), 1468, 1449, 1366, 1274, 1239; FAB-MS (positive mode) m/e 829 (M + H)$^+$. Anal. Calcd for $C_{42}H_{44}N_4O_{12}S{\cdot}H_2O$: C, 59.57; H, 5.47; N, 6.61. Found: C, 59.63; H, 5.46; N, 6.48.

(17) **Preparation of 5.** A solution of **4a** (200 mg) in methanol containing 10% Pd–C (20 mg, 10 mL) was stirred under hydrogen at atmospheric pressure for 16 h at ambient temperature after which time TLC showed the complete disappearance of starting material. The suspension was filtered through a pad of Celite, the filtrate evaporated, and the resulting solid crystallized from a $CHCl_3$–hexane mixture to yield 140 mg (72%) of a fine white solid which melted from 198 to 200 °C: ^{1}H NMR (Me_2SO-d_6) δ 1.50 (s, 3 H, CH_3), 1.76 (s, 3 H, CH_3), 1.82–1.96 (m, 2 H, 5''-CH_2), 2.01–2.08 (m, 1 H, 2''-CH_2), 2.16–2.34 (m, 3 H, 5''-CH, 2''-CH, and 2'-CH), 2.40–2.54 (m, 1 H, 2'-CH), 3.03–3.03 (t, 2 H, O_2SCH_2), 3.24–3.28 (m, 2 H, 5'-CH_2), 3.29–3.36 (m, 1 H, 4''-CH), 3.44–3.54 (m, 1 H, 3''-CH), 3.88–3.94 (m, 1 H, 4'-CH), 4.10–4.18 (q, 1 H, 3'-CH), 6.07–6.10 (t, 1 H, 1''-CH), 6.12–6.19 (t, 1 H, 1'-CH), 7.14–7.43 (m, 16 H, trityl H and 6 H), 7.52 (s, 1 H, 6 H), 11.60 (bs, 1 H, NH); ^{13}C NMR (Me_2SO-d_6) δ 11.7, 11.9 (2 CH_3's), 26.7 (5''-C), 37.5 (2'-C), 38.3 (2''-C), 49.1 (O_2SCH_2), 52.0 (3'-C), 54.3 (3''-C), 62.6 (5'-C), 82.6, 82.8, 83.1, 83.3 (4'-C, 4''-C, 1'-C, 1''-C), 86.2 (trityl C), 109.3, 109.7 (two 5-C's), 126.9, 127.7, 128.1 (aromatic C's), 135.6, 136.0 (two 6-C's), 143.2 (aromatic C), 150.1, 150.2 (two 2-C's), 163.5 (two 4-C's); IR (KBr) cm^{-1} 3350 b (NH), 3440 b (NH), 1691 bs (CO), 1469, 1449, 1271, 1141; FAB-MS (positive mode) m/e 784 (M + H)$^+$. Anal. Calcd for $C_{40}H_{44}N_6O_9S{\cdot}1.5H_2O$: C, 59.20; H, 5.79; N, 10.35. Found: C, 59.28; H, 5.83; N, 10.12.